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Assessing the ecological effects of water stress and pollution in a temporary river - Implications for water management

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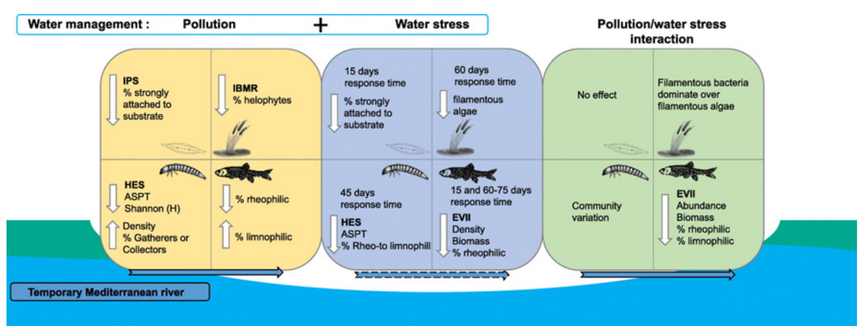
HIGHLIGHTS

- Pollution and water stress effects on communities and metrics were examined
- Pollution mostly affected diatoms, macrophytes and macroinvertebrates
- Water stress and the combined effect of the two stressors mostly affected fish
- Different temporal effects of water stress were observed on the four biotic groups
- Management decisions should be based on both pollution and temporal water stress data

GRAPHICAL ABSTRACT



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ABSTRACT

Temporary rivers are dynamic and complex ecosystems that are widespread in arid and semi-arid regions, such as the Mediterranean. Biotic communities adapted in their intermittent nature could withstand recurrent drought events. However, anthropogenic disturbances in the form of water stress and chemical pollution challenge biota with unpredictable outcomes, especially in view of climate change. In this study we assess the response of the biotic community of a temporary river to environmental stressors, focusing on water stress and pollution. Towards this aim, several metrics of four biotic groups (diatoms, macrophytes, macroinvertebrates and fish) were applied. All biotic groups responded to a pollution gradient mainly driven by land use, distinct functional groups of all biota responded to water stress (a response related to the rheophilic nature of the species and their resistance to shear stress), while the combined effects of water stress and pollution were apparent in fish. Biotic groups presented a differential temporal response to water stress, where diatom temporal assemblage patterns were explained by water stress variables of short-time response (15 days), while the responses of the other biota were associated to longer time periods. There were two time periods of fish response, a short (15 days) and a long-time response (60–75 days). When considering management decisions, our results indicate that, given the known response of river biota to pollution, biomonitoring of temporary rivers should also involve metrics that can be utilized as early warnings of water stress.

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1. Introduction

Research on temporary rivers has vastly increased during the last decade (Datry et al., 2011, 2014; Acuña et al., 2014; Skoulikidis et al., 2017). Temporary streams and rivers, are among the most dynamic, complex and diverse freshwater ecosystems, but also among the most threatened ecosystems (Larned et al., 2010; Acuña et al., 2014). Though located in all geographical regions, they are by far the dominant river type in arid and semi-arid areas (McDonough et al., 2011; Acuña et al., 2014). Temporary rivers occupy more than one third of the planet's land surface and host about 30% of the world population (Tzoraki and Nikolaidis, 2007; Arenas-Sánchez et al., 2016). They are vulnerable to natural drought and anthropogenic water stress (Larned et al., 2010; Acuña et al., 2014); in most European Mediterranean rivers, the combination of extensive water abstraction, river fragmentation, and climate change has dramatically reduced river runoff (UNEP/MAP, 2003). The Intergovernmental Panel on Climate Change (IPPC) predicts that the trend of increasing temperatures and reduced annual precipitation will lead to prolonged drought events (IPCC Core Writing Team et al., 2014). Mediterranean temporary rivers encompass a remarkable hydrogeomorphological diversity, as well as a unique native fauna, adapted to natural drought events (Gasith and Resh, 1999; Lake, 2003; Matthews and Matthews, 2003), but are sensitive to the disturbance caused by the anthropogenic increase in the frequency and severity of water stress episodes (Magalhães et al., 2007; Datry et al., 2014). Mediterranean river basins are becoming drier (annual precipitation decreased up to 20% during the 20th century), with more extreme events than a century ago (García-Ruiz et al., 2011). Moreover, the recent drought that began in 1998 in the eastern Mediterranean Levant region, is likely the worst drought of the past nine centuries, as concluded by a recent NASA (National Aeronautics and Space Administration) study, aiming to reconstruct the Mediterranean's drought history (Cook et al., 2016). All the above bring temporary rivers to the spotlight in view of climate change and its effects on river communities.

Mediterranean temporary rivers are subjected, apart from water stress, also to nutrient enrichment from industrial and urban wastewaters, and organic pollution from agricultural activities (Meybeck, 2004; Vörösmarty et al., 2010). Water intermittency can accentuate the effects of pollution stressors by affecting the dilution and self-purification capacity of the receiving aquatic systems (Karaouzas et al., 2011; Sabater et al., 2016). Furthermore, high seasonal flow fluctuation due to natural variation and over-abstraction, influences the physicochemical and biological characteristics of these ecosystems (Barceló and Sabater, 2010; Arenas-Sánchez et al., 2016; Skoulikidis et al., 2017; Kalogianni et al., 2017). The absence of long-term biological data related to environmental data at large spatial scales in temporary rivers has so far limited the understanding of biota responses to single and multiple pressures.

The effects of chemical and water stress on biotic communities appear to be both complex and temporally and spatially variable (Arenas-Sánchez et al., 2016; Sabater et al., 2016). Aggregates of organisms such as biofilm, macrophytes, invertebrates, or fish have specific responses to various stressors, related to their habitat requirements and life-cycle (Sabater et al., 2007; Johnson and Hering, 2009; Johnson and Ringler, 2014). The joint exposure to pollution and water stress may produce cumulative impacts on the aquatic biotic assemblages, as they are subjected to a concomitant habitat shrinkage, water quality deterioration and increased competition for limited resources (Magalhães et al., 2002; Magoulick and Kobza, 2003). Pollution and water stress can have pronounced effects on macroinvertebrate and fish species richness, abundance and community structure (Larned et al., 2010; Petrovic et al., 2011; Arenas-Sánchez et al., 2016; Kalogianni et al., 2017). On the other hand, the response of macrophytes and benthic diatoms to multiple stressors in temporary ecosystems is much less studied. There is still a large uncertainty on how hydrological variation and chemical pollution affect aquatic communities under varying environmental conditions and how co-occurring stressors affect the community structure of the various freshwater biota (Navarro-Ortega et al., 2014; Sabater et al., 2016).

The aim of this study was to assess the response of the biotic community of a temporary river to environmental stressors, focusing on water stress and pollution. The community under study consisted of four biotic groups, namely benthic diatoms, macrophytes, benthic macroinvertebrates and fish. To test the effects of the different stressors, we applied two approaches, retaining different information levels. We first investigated for stressors' effects and their interactions in metrics commonly used for each biotic group, including biological quality and diversity indices. We then applied a community-based approach where we considered the effects of the stressors for the whole community of each biotic group. Considering the physiological differences of the four biotic groups used in the present study, we also hypothesized that their reaction to water stress would be time dependent. We expected that our integrative approach would lead to a deeper understanding of communities in temporary rivers and would further inform and enhance management decisions.

2. Materials and methods

2.1. Study area

This study was conducted in the Evrotas River (36°48′15″N–22°41′45″E, Southern Peloponnese, Greece, Fig. 1). The Evrotas basin is representative of a large fraction of Greek territory drained by temporary rivers (up to 43%, Tzoraki and Nikolaidis, 2007). It is a medium-sized (2.418 km^2), mid-altitude (150–600 m) Mediterranean basin, with several ephemeral and intermittent streams discharging into the main channel of the river (Vardakas et al., 2015; Karaouzas et al., 2017). The climate, discharge and precipitation of the Evrotas basin follow a predictable seasonal pattern, similar to other Mediterranean rivers (Gasith and Resh, 1999) with hot and dry summers and cool, wet winters. However, flow intermittency in Evrotas is highly dependent on the hydrological/meteorological conditions of the preceding year(s) that inevitably also affect the intensity of water abstractions. Water abstraction for field irrigation is the dominant anthropogenic pressure in the Evrotas River Basin leading to the artificial desiccation of several sections in late summer-early autumn (Skoulikidis et al., 2011). The geographical, geological, hydrological and ecological features of the Evrotas watershed are described in detail elsewhere (Karaouzas et al., 2017; Kalogianni et al., 2017; Vardakas et al., 2017a).

Samplings were conducted at four sites on the main channel of Evrotas River in June 2014, July 2015, June 2016 and September 2016 targeting for different levels of water stress and water quality degradation (Fig. 1). The two upstream sites (Uskol and Dskol) are relatively undisturbed and typologically similar, with Uskol located in a perennial river section, while Dskol located 1.2 km downstream, is an intermittent site drying out partially during late summer (Fig. 1). The other two sites (Vivari and WWTP) are located in the middle section of the Evrotas River with a wider active channel and higher discharge than the upstream sites; the spring-fed Vivari is a relatively undisturbed perennial site, while the WWTP site, located 20 km downstream, dries out in periods of extreme drought. Vivari receives diffuse agricultural pollution and minor pollution from animal husbandry (Fig. 1). The heavily impacted WWTP receives diffuse pollution from agriculture and point source pollution from the Sparta Wastewater Treatment Plant and from cesspool waste dumping, plus seasonal pollution from olive mill and orange juice processing wastewaters.

2.2. Environmental data collection and biota sampling

Water physicochemical parameters, i.e. dissolved oxygen (D.O. mg/L), water temperature (°C), conductivity (μS cm^{-1}), were measured at each site using a Portable multiparameter Aquaprobe AP-200 with a GPS Aquameter (Aquaread AP 2000). Water samples for nutrient

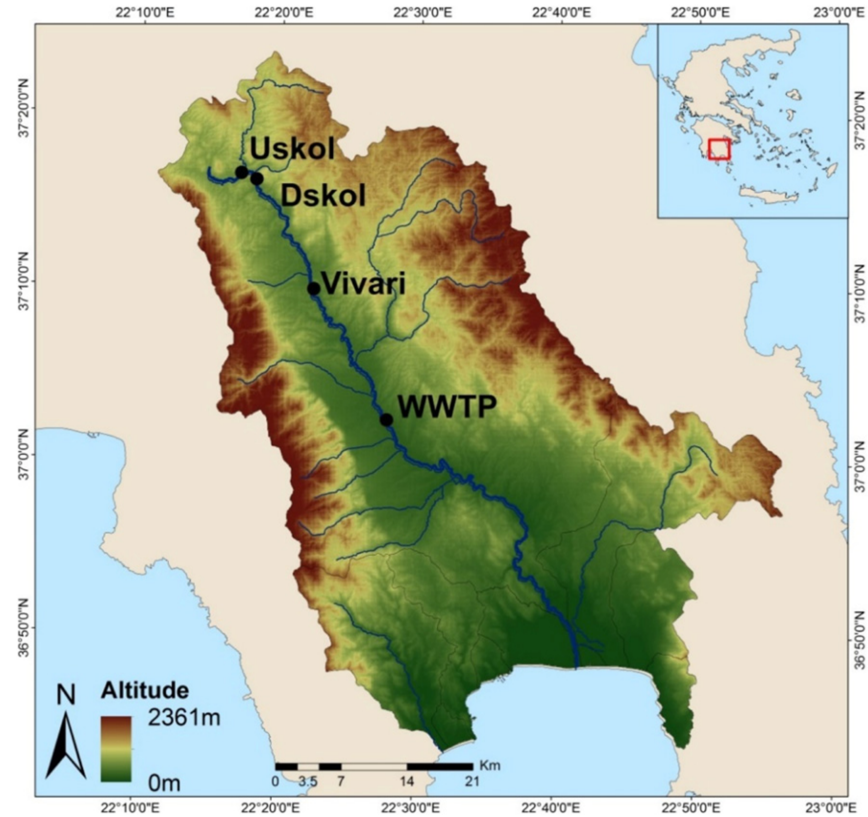

Fig. 1. The Evrotas river catchment and the four sampling sites.

analyses were filtrated through 0.45 μm membrane filters. Nitrite (NO_2, mg/L), and orthophosphate (PO_4, mg/L) concentrations were determined by a Skalar San++ Continuous Flow Analyzer (Boltz and Mellon, 1948; Navone, 1964), whereas nitrate (NO_3, mg/L) concentration was determined using both Ion Chromatography and a Skalar Automatic Analyzer. NH_4, mg/L concentration was determined using a Skalar Automatic Analyzer.

Water flow data were obtained during sampling using a Water Flow meter OTT C20, while discharge was calculated based on the velocity - area method (Buchanan and Somers, 1976). Land use classes distribution of the Evrotas River basin was based on CORINE 2012 database (European Environmental Agency, 2012).

2.2.1. Diatom sampling

Sampling of benthic diatoms followed the standard "EN 13946 (European Committee for Standardization, 2003a): Water quality. Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers". During sampling, 5–7 submerged cobbles were collected from lit areas and brushed to obtain biofilm material. In the laboratory, samples were digested using the hot hydrogen peroxide method, to remove any organic matter. Permanent slides were prepared with Naphrax. Up to 400 valves were counted and identified at the species or subspecies level in each sample, using light microscopy (Nikon Eclipse 80i, Tokyo, Japan) with Nomarski differential interference contrast optics at 1000× magnification.

2.2.2. Macrophyte sampling

Macrophytes were surveyed following the standard "EN 14184 (European Committee for Standardization, 2003b): Methods for surveying aquatic macrophytes in running and standing waters". The survey was performed in a 100 m stretch of the river by wading upstream, following a meandering route. The presence of aquatic macrophytes was recorded by estimating the percentage cover of each species on a 1–5 scale (1: <0.1%; 2: 0.1–<1%; 3: 1–<10%; 4: 10–<50%; 5: ≥50%). Samples of macrophytes were transported to the laboratory for further determination.

2.2.3. Macroinvertebrate sampling

Macroinvertebrate collection followed the STAR-AQEM methodology (AQEM Consortium, 2002). During the current study, 20 'replicates' i.e. subsamples, were obtained at each sampling site. Benthic macroinvertebrates were collected using a 25 cm × 25 cm square hand net with a mesh size 500 μm nytex screen. Each of the 20 subsamples was taken by positioning the net and disturbing the substrate in an area that equals the square of the frame width upstream of the net (25 × 25 cm). Thus, a total of 1.25 m^2 (25 × 25 × 20 replicates) was sampled from each sampling site. Subsamples were preserved in ethanol until transportation to the laboratory where they were sorted and identified. All macroinvertebrates were identified to family level and, where possible, to genus or species level. However, family level was used for statistical analysis in order to have a homogeneous dataset.

2.2.4. Fish sampling

Fish samplings were conducted using an EFKO electrofishing DC unit (Honda 7k VA generator, 150 m cable, 1.5 m anode pole, 6A DC output, voltage range 300–600 V); for a detailed description of the sampling method see Vardakas et al. (2017a). Briefly, at all samplings, the team consisted of the same four operators to ensure consistency of sampling effort. Electrofishing began at a shallow riffle and proceeded upstream in a meandering manner to adequately sample all types of habitats. Captured fish were identified to species level, counted, their size class recorded at 5 cm intervals, and then they were returned alive to the river. Fished length varied from 250 to 300 m and was consistent among samplings at each site.

2.3. Data analysis

2.3.1. Abiotic data

The hydrological variables, used as indicators of water stress, included discharge, discharge viability (i.e. the coefficient of variation of discharge in time), the mean duration of low spells (in days) and the number of days with zero discharge. To define the levels of water stress during samplings, a general linear model using measured discharge at all sites was performed, with samplings as a 4-level factor (corresponding to the four sampling campaigns); Duncan post hoc test was applied to test for significant differences. Viability of discharge, mean duration of low spells and the number of days with zero discharge, were based on simulated discharge outputs of the model SWAT (Neitsch et al., 2011) developed by Gamvroudis (2016) for the Evrotas river basin, due to the lack of long term and detailed daily discharge measurements in the study area. The tool used for the hydrological analysis at Evrotas sampling sites was the Time Series Analysis (TSA) module of River Analysis Package (RAP) v 3.0.7, developed by the Cooperative Research Centre for Catchment Hydrology (CRCCH) of Australia (Marsh, 2004). The hydrological indicators were calculated for the periods of 15, 30, 45, 60, 75 and 90 days prior to each sampling date for each sampling site. Land use, physico-chemical and chemical variables were either log or arcsin (proportions) transformed prior to use. Pearson's correlation analysis was performed with the transformed data (separately for the hydrological and environmental variables), and highly correlated variables (>80%) were excluded.

2.3.2. Effect of water stress and pollution on biotic metrics

Biotic metrics used (listed in Table 2) included biological quality indices, diversity indices and proportions of specific functional groups in each assemblage. Biological quality indices applied to the National water quality assessment included the IPS (Specific Pollution Sensitivity) index (CEMAGREF, 1982) for diatoms, the IBMR index (Haury et al., 2006) adjusted for Greece (IBMR_GR, Aguiar et al., 2014; Papastergiadou, 2015) for macrophytes, and the HES index for macroinvertebrates (Artemiadou and Lazaridou, 2005). For fish, a spatially based multimetric index, developed specifically for the Evrotas River, was applied (EVII, Skoulikidis, 2008). Diversity indices included species richness, Shannon diversity (Shannon and Weaver, 1949), Pielou's evenness (Pielou, 1975) and dominance indices. Functional groups considered, included the diatoms potential ability to resist water flow (Liu et al., 2013), the macrophyte floristic groups affinity to water (Birk et al., 2007), the current and microhabitat preference and feeding guilds for macroinvertebrates (Juhász, 2016) and structural and functional traits for fish (Skoulikidis, 2008).

Hydrological variables defined two levels of water stress: low water stress (LWS) during the 2014 and 2015 samplings and high water stress (HWS) during the two 2016 samplings ($F_{3,57} = 7.26$, $p < 0.001$, Fig. 2a). Principal Component Analysis (PCA) using all the environmental variables was performed in order to summarize the total variability of the data. The first component (PC1) accounted for 49% of the total variance and was used as a summary variable of pollution (Fig. 2b). Variables contributing most in PC1 were dissolved oxygen (DO), urbanization, K^+, PO_4^{2-}, NH_4^+, conductivity, and water temperature.

A general linear model (GLM) with one categorical and one numerical variable was applied to test for the effect of the two stressors on each biotic metric (Table 2). The significance of their interaction was used as an indicator of an existing combined effect. The general formula for the GLM was:

$$\text{Biotic metric} \sim \text{WSL} + \text{PC1} + \text{WSL} : \text{PC1}$$

where, WSL is the water stress level (2 levels-HWS, LWS), PC1 are the scores of PC1 summarizing environmental variables and WSL:PC1 is there interaction. The F-statistic and its associated p-value were used as a measure of significance of effects.

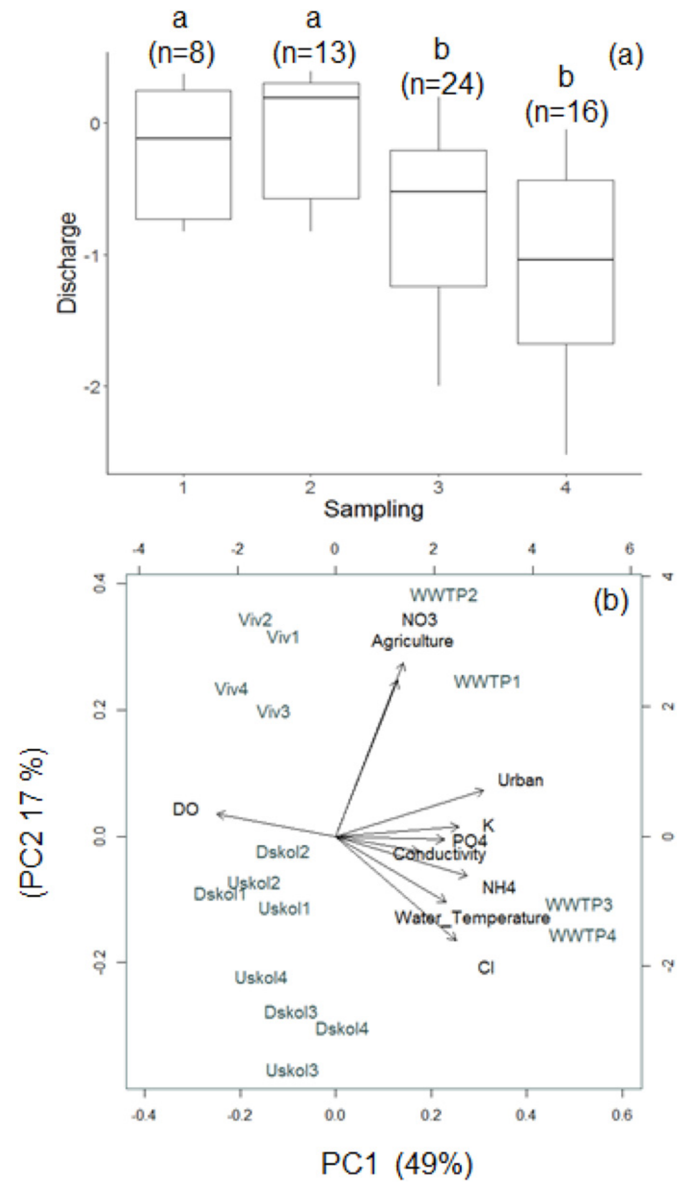

Fig. 2. Definition of water stress levels and summary of environmental variables. (a) Boxplot of discharge during the four sampling campaigns; a and b show the group each sample belongs in based on Duncan post hoc test, n is the number of replicates, data were log10 transformed, y axis is in log10 scale. (b) PCA with environmental variables. Percentage of variance explained by each PC is in parenthesis. In gray are the 4 sites; Numbers 1–4 next to the site name indicate the 4 sampling campaigns (June 2014, July 2015, June 2016 and September 2016). Scaling on top and right axis correspond to the environmental variables (arrows).

2.3.3. Effect of water stress and pollution on community matrices

The ordination of community composition with respect to hydrological and environmental variables was performed by means of a Redundancy Analysis (RDA). Redundancy analysis is a constrained ordination method, which can be thought of as an extension of multiple regression, applied to multiple response data (Šmilauer and Lepš, 2014). Biotic data were Hellinger transformed (i.e. square root transformation of relative abundances) to allow the use of Euclidean-based approaches such as the RDA (Legendre and Gallagher, 2001). Furthermore, Detrended Correspondence Analysis (DCA) provided a gradient length of the first DCA axis smaller than 3 times Standard Deviation in all biotic groups, thus confirming the use of RDA as the most appropriate ordination method. Forward selection of environmental variables was used to ascertain the minimal set of variables that explain species data. Together

with measured discharge, hydrological variables of simulated discharge of the 15-days' time series were used, as the one closer to sampling date. Considering fish, two size classes (<10 cm and >10 cm) of *Squalius keadicus* (Evrotas chub) were maintained separately in these analyses, since previous studies have documented differences in the habitat requirements of juvenile and adult chubs (Vardakas et al., 2017a).

Variation in each community matrix was partitioned into components accounting for the hydrological variables (indicative of water stress), the environmental variables (indicative of pollution), and their shared effects (Borcard et al., 1992). Together with measured discharge, hydrological variables of simulated discharge of the 15-days' time series were used, as the one closer to sampling date. Variation partitioning aims in determining the likelihood of the above sets of predictors in explaining patterns in biotic community structure. It should be noted that the shared partition is not an interaction term but rather a correlation term, showing the community variation that can be explained by both explanatory matrices. Therefore, increased shared effects render the analysis inconclusive in explaining patterns. The function applied uses adjusted R squares to assess the partitions explained by the explanatory group of variables and their combinations, as it corrects for the number of variables in each group and is thus considered an unbiased method (Peres-Neto et al., 2006).

2.3.4. Temporal differential effects of water stress

Time effects of water stress on each biotic group, were assessed using variation partitioning (Borcard et al., 1992). The environmental variables (indicative of pollution) were held constant and the hydrological variables (indicative of water stress) were changing. Specifically, model outputs corresponding to each time series (from 15 to 90 days) were applied separately, together with measured discharge. This allowed isolating and tracking any differences of the effect of water stress in explaining community variation.

Data analysis was performed in R v.3.3.3 (R Core Team, 2017). For multivariate analysis, packages vegan v.2.4.2 (Oksanen et al., 2017) and packfor (Dray et al., 2016) were used.

3. Results

Land use at the two upstream sites (Uskol, Dskol) is dominated by forest area, whereas agriculture is the main land use in Vivari and WWTP (Table 1). Urbanization appears only in the WWTP site. Overall the WWTP site, downstream of the Sparta Wastewater Treatment Plant, was the most polluted, as indicated by numerous physicochemical variables (Table 1). NO_2, NH_4^+ and K^+ were always higher in the WWTP site compared to the other sampling sites, irrespective of water stress level. On the other hand, Cl^-, conductivity and water temperature were higher in WWTP compared to the other sampling sites at high water stress, while DO presented the lowest concentration. PO_4^{3+} and NO_3^- concentrations were higher in the WWTP site compared to the other sampling sites at low water stress. NO_3 concentration and conductivity also seem to follow the river gradient, presenting lower values in the two upstream sites and higher in the downstream sites.

Pearson's correlation analysis showed that of all the environmental variables used (K^+, Cl^-, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, agriculture, urbanization, forest, dissolved oxygen (DO), conductivity, water temperature), forest area was highly correlated with the extent of agriculture, and NO_2 concentration was highly correlated with urbanization and NH_4 concentration. Therefore, forest area and NO_2 concentration were removed from further analysis.

3.1. Effect of water stress and pollution on biotic metrics and biological functional traits

Diatoms responded more to pollution levels based on the biological quality index (IPS), which decreased with increasing pollution, irrespective of water stress levels (Table 2, Fig. 3a). Both pollution and water stress had a negative effect on the proportion of the species strongly attached to the substrate. However, the two stressors did not affect the response of each other in any way, as indicated by the non-significant interaction term (Table 2, Fig. 3b).

Macrophytes responded more to pollution levels based on the biological quality index (IBMR_GR), which decreased with increasing pollution, at both water stress levels (Table 2, Fig. 3c). This was also the case for the proportion of helophytes. On the other hand, the proportion of filamentous bacteria colonizing the streambed was affected by both stressors and their interaction. Filamentous bacteria were only present in the most impacted WWTP site at high water stress, accounting for 50% of total coverage. Filamentous algae presented an increase with pollution at low water stress and a decrease when the two stressors acted in combination (Table 2, Fig. 3d).

Macroinvertebrates responded overall more on pollution gradients rather than water stress levels (Table 2). However, the biological quality index for this biotic group (HES) also responded to water stress levels by decreasing at high water stress (Fig. 4a). Total density (abundance of individuals m^{-2}) increased with increasing pollution (Fig. 4b). A decrease in diversity (Shannon index, Fig. 4c) and an increase in the dominance of gatherers/collectors at increasing pollution were also observed. Average score per taxon (ASPT) decreased with pollution and water stress increase (Fig. 4d). High water stress decreased the proportion of rheo- to limnophill taxa (taxa that prefer slowly flowing streams and lentic

Table 1
Mean ± SD of environmental data (physicochemical and land use) of the four sites during low (LWS) and high (HWS) water stress. Land use did not change during the samplings. There were two samplings (n = 2) at every water stress level.

		Uskol		Dskol		Vivari		WWTP	
		LWS	HWS	LWS	HWS	LWS	HWS	LWS	HWS
Physicochemical variables	K^+ (mg/L)	0.79 ± 0.028	0.775 ± 0.035	0.41 ± 0.453	0.89 ± 0.198	0.72 ± 0.028	0.64 ± 0.028	1.8 ± 0.919	2.365 ± 0.092
	Cl^- (mg/L)	8.75 ± 0.014	11.15 ± 0.269	9.015 ± 0.559	11.215 ± 0.757	7.285 ± 0.318	9.2 ± 0.184	10.575 ± 0.049	16.08 ± 3.168
	NO_3^- (mg/L)	0.6 ± 0.014	0.37 ± 0.028	0.57 ± 0.071	0.315 ± 0.021	0.86 ± 0.028	0.61 ± 0.042	1.415 ± 0.247	0.72 ± 0.014
	NO_2^- (mg/L)	0.0015 ± 0.001	0.0015 ± 0.001	0.002 ± 0.000	0.0025 ± 0.001	0.0015 ± 0.001	0.002 ± 0.000	0.0345 ± 0.037	0.0725 ± 0.046
	NH_4^+ (mg/L)	0.012 ± 0.001	0.0085 ± 0.002	0.0105 ± 0.004	0.032 ± 0.014	0.011 ± 0.001	0.0095 ± 0.004	0.043 ± 0.044	0.157 ± 0.130
	PO_4^{3-} (mg/L)	0.002 ± 0.001	0.0025 ± 0.001	0.001 ± 0.000	0.00695 ± 0.009	0.001045 ± 0.001	0.002 ± 0.000	0.034 ± 0.025	0.0105 ± 0.006
	DO (mg/L)	9.35 ± 0.212	10.07 ± 0.990	9.33 ± 0.467	9.715 ± 0.233	9.3 ± 0.141	10.13 ± 0.212	9.05 ± 0.212	4.495 ± 2.128
	Conductivity (μS/cm)	431 ± 48.083	488 ± 43.841	408.5 ± 58.690	432 ± 21.213	509.5 ± 77.075	329.5 ± 269.408	559 ± 65.054	637.5 ± 19.092
	Water temperature	19.3 ± 1.556	19.9 ± 4.667	21 ± 0.131	20.3 ± 1.273	18.9 ± 0.141	17.65 ± 1.061	20.9 ± 1.131	24.45 ± 1.485
Land use	%Forest	80	80	60	60	30	30	10	10
	%Agriculture	20	20	40	40	70	70	65	65
	%Urban	0	0	0	0	0	0	15	15

Table 2
Effects of water stress (two-level factor), pollution (as summarized in the PC1) and their interaction on tested metrics for the four biotic groups, resulted from the respective general linear models. Functional groups of macroinvertebrates are further grouped based on current preference, microhabitat preference and feeding guilds as defined in Juhász (2016).

		Water stress (df = 1)	Pollution (df = 1)	Interaction Water stress-Pollution (df = 1)
Biotic group	Metric	F-value	F-value	F-value
Diatoms	IPS (Ecological Quality Index)	0.75	29.47***	0.08
	Species richness (S)	0.71	0.34	2.59
	Shannon diversity (H′)	0.44	0.70	2.83
	Pielou evenness (J′)	0.01	1.16	2.26
	% Strongly attached to substrate	10.98**	9.98**	0.60
Macrophytes	IBMR_GR (Ecological Quality Index)	0.93	30.64***	0.00
	Species richness (S)	1.30	2.86	0.45
	Shannon diversity (H′)	2.40	0.31	3.19
	% Filamentous algae	0.24	2.57	12.44**
	% Filamentous bacteria	40.54***	157.87***	73.45***
	% Bryophytes	0.13	1.02	0.02
	% Hydrophytes	2.66	0.11	0.03
	% Helophytes	1.39	5.41*	1.18
	% Hygrophytes	0.05	0.12	0.16
Macroinvertebrates	HES (Ecological Quality Index)	7.38*	52.59***	1.57
	Density (ind/m^2)	1.07	67.40***	0.78
	Taxa richness (S)	2.01	2.74	3.51
	Shannon diversity (H′)	0.18	17.43**	2.59
	r-Dominance	0.41	5.79*	0.61
	Average score per Taxon (ASPT)	10.75**	82.37***	0.55
	BMWP Score (Greek version)	2.36	9.14*	3.75
	r/K relationship	0.41	0.08	1.63
	Number of sensitive taxa (Austria)	2.15	12.53**	0.16
	% EPT Taxa	2.69	3.27	0.80
	EPT/Diptera	1.71	8.71*	0.31
	%OCH	1.87	5.58*	0.31
	Traits related to current preference			
	% Limnobiont (LB)	0.01	0.31	3.80
	% Limnophill (LP)	0.47	1.15	0.10
	% Limno- to rheophill (LR)	1.72	14.52**	1.24
	% Rheo-to limnophill (RL)	6.47*	1.35	0.03
	% Rheophill (RP)	0.43	6.43*	0.67
	% Rheobiont (RB)	0.13	0.69	0.04
	% Indifferent (IN)	0.01	7.74*	0.64
	Traits related to microhabitat preference			
	% Pelal (PEL)	0.18	5.48*	1.03
	% Psammal (PSA)	0.49	1.59	0.05
	% Akal (AKA)	0.01	6.90*	0.29
	% Lithal (LIT)	0.72	5.59*	0.20
	% Phytal (PHY)	3.09	0.11	0.72
	% Particular Organic Matter (POM)	0.05	4.65	0.47
	% Other habitats (OTH)	0.24	5.90*	1.03
	Traits related to feeding guild			
	% Grazers and scrapers	0.21	3.12	0.00
	% Miners	0.08	6.49*	0.19
	% Shredders	0.27	4.76	0.16
	% Gatherers or Collectors	12.79**	6.04*	0.44
	% Passive filter feeders	1.86	4.06	0.21
	% Predators	1.42	0.18	0.00
	% Parasites	0.25	6.00*	1.05
Fish	EVII (Ecological Quality Index)	6.43*	0.17	5.10*
	Density (ind/Fished area m^2)	6.69*	0.16	3.55
	Density (ind/volume m^3)	10.33**	2.38	2.40
	Total abundance	0.11	3.23	16.21**
	Total Biomass (gr)	10.91**	0.47	18.68***
	% *S.keadicus* (Rheophilic larger-bodied)	4.90*	5.25*	4.80*
	% *S. keadicus* > 10 cm	4.59	0.94	0.14
	% *T. spartiaticus* (Limnophilic)	1.14	44.51***	19.55**
	% *P. laconicus* (Limnophilic smaller-bodied)	12.90**	1.82	0.00

Residual df = 12.
* Significance level < 0.05.
** Significance level < 0.01.
*** Significance level < 0.001.

zones - Table 2 and Fig. 4e); whereas other groups were only affected by pollution, with the proportion of rheophill taxa decreasing and that of limno - to rheophill taxa increasing. Pollution affected also the distribution of individuals based on their microhabitat preference (Table 2), decreasing the ones that prefer gravel (akal and lithal substrate) while increasing the ones that prefer mud (pelal substrate). An increase in the abundance of miners, parasites and detritus feeders (i.e. gatherers or collectors), was observed as pollution increased (Table 2). Detritus feeders in particular were also affected by water stress, with lower densities at higher water stress (Fig. 4f). The two stressors (pollution and water stress) did not produce an interaction effect on macroinvertebrate metrics.

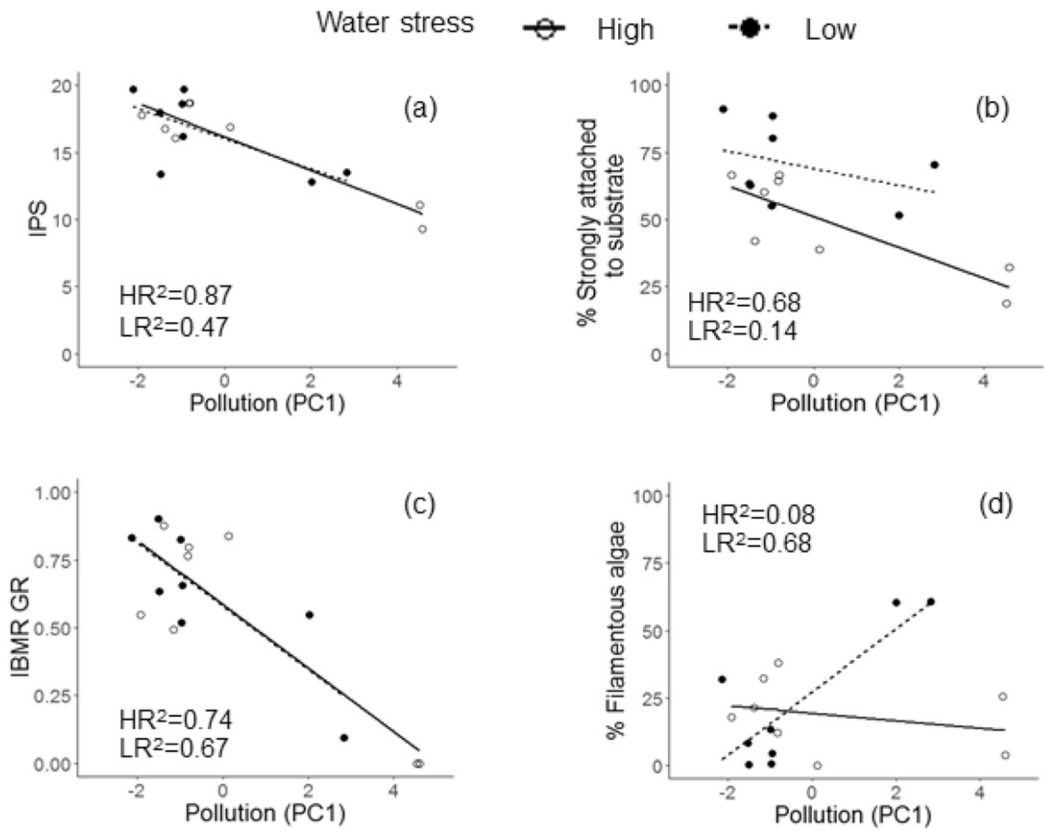

Fig. 3. Graphical representation of trends of selected biotic metrics of primary producers (a,b - diatoms, c,d -macrophytes) that significantly responded to water stress and pollution (Table 2 for statistics). IPS and IBMR GR are the biological quality indices for the two groups. Solid line and open circles correspond to high water stress, dashed line and filled circles correspond to low water stress. R^2 are only indicative of the fit of each regression line to the corresponding data. HR^2 corresponds to high water stress line and LR^2 to low water stress line.

Fish mostly responded to water stress (Table 2) whereas their response to pollution depended on water stress levels (significant interaction terms in Table 2). The fish biotic index (EVII) decreased with increasing water stress, with increasing pollution at high water stress enhancing this negative effect (Fig. 5a). Fish densities, increased at high water stress and decreased with pollution (Fig. 5b). Total abundance and biomass increased at low water stress with increasing pollution, whereas, at high water stress, it decreased with increasing pollution (Fig. 5c, d). The rheophilic species *Squalius keadicus* decreased, at low water stress, with increasing pollution, whereas at high water stress it contributed less to the total abundance along the pollution gradient (Fig. 5e). The limnophilic *Tropidophoxinellus spartiaticus* followed the pollution gradient at low water stress, while similarly to *S. keadicus*, its abundances were reduced at high water stress along the pollution gradient (Fig. 5f). Only the small bodied, limnophilic *Pelasgus laconicus* presented higher abundances at high water stress. Pollution and water stress presented an interaction effect to all fish metrics examined, with the exception of fish density and the percentage of *P. laconicus* (Table 2).

3.2. Effect of water stress and pollution on community matrices

Diatom assemblages were affected by both environmental and hydrological variables that accounted for 66% of the total variation in the RDA analysis (Fig. 6a). Urbanization and low DO levels shaped assemblages in the most polluted WWTP site. Hydrological variables constrained the assemblages in Vivari and in Dskol and Uskol sites at low water stress. Both the environmental (indicative of pollution) and the hydrological (indicative of water stress) variables explained diatom assemblage patterns, with the environment playing a more important role, as indicated by variation partitioning analysis (Fig. 7a).

Macrophyte communities were shaped by environmental variables that explained a low portion of variation (42%), based on land use (extend of agriculture and urbanization) and Cl^- concentration, indicative of KCl fertilizers or urea pollution. Increased urbanization and Cl^- concentration characterized the macrophyte community in the most polluted WWTP site, whereas low agriculture levels shaped the community of Uskol and Dskol (Fig. 6b). Greater variation of the macrophyte community patterns was explained by environmental variables (indicative of pollution) than by water stress, with the shared variation being very low (Fig. 7b).

Macroinvertebrate communities were shaped by both environmental and hydrological variables that explained 69% of total variation (Fig. 6c). This was also reflected in the large shared variation between pollution and water stress in explaining macroinvertebrate community patterns, introducing an uncertainty between the real contribution of each explanatory group of variables (Fig. 7c). High water temperature and urbanization as well as small changes in discharge shaped the communities in the WWTP site, whereas agriculture and NO_3 concentrations shaped communities in Dskol and Uskol sites.

Fish assemblages were mainly driven by land use (extent of agriculture and urbanization), DO and Cl^- concentrations in a total variation of 86% (Fig. 6d). Increased urbanization as well as decreased DO shaped fish assemblages in the most polluted WWTP site whereas low agriculture levels shaped the community of Uskol and Dskol. Pollution had the highest contribution in explaining patterns of fish assemblages, whereas explained variation by water stress was the highest among biotic groups and unexplained variation the lowest (only 2%, Fig. 7d).

3.3. Temporal differential effects of water stress

Diatom assemblages presented a short-time response of 15 days to water stress variables (Table 3). In longer time periods, both water

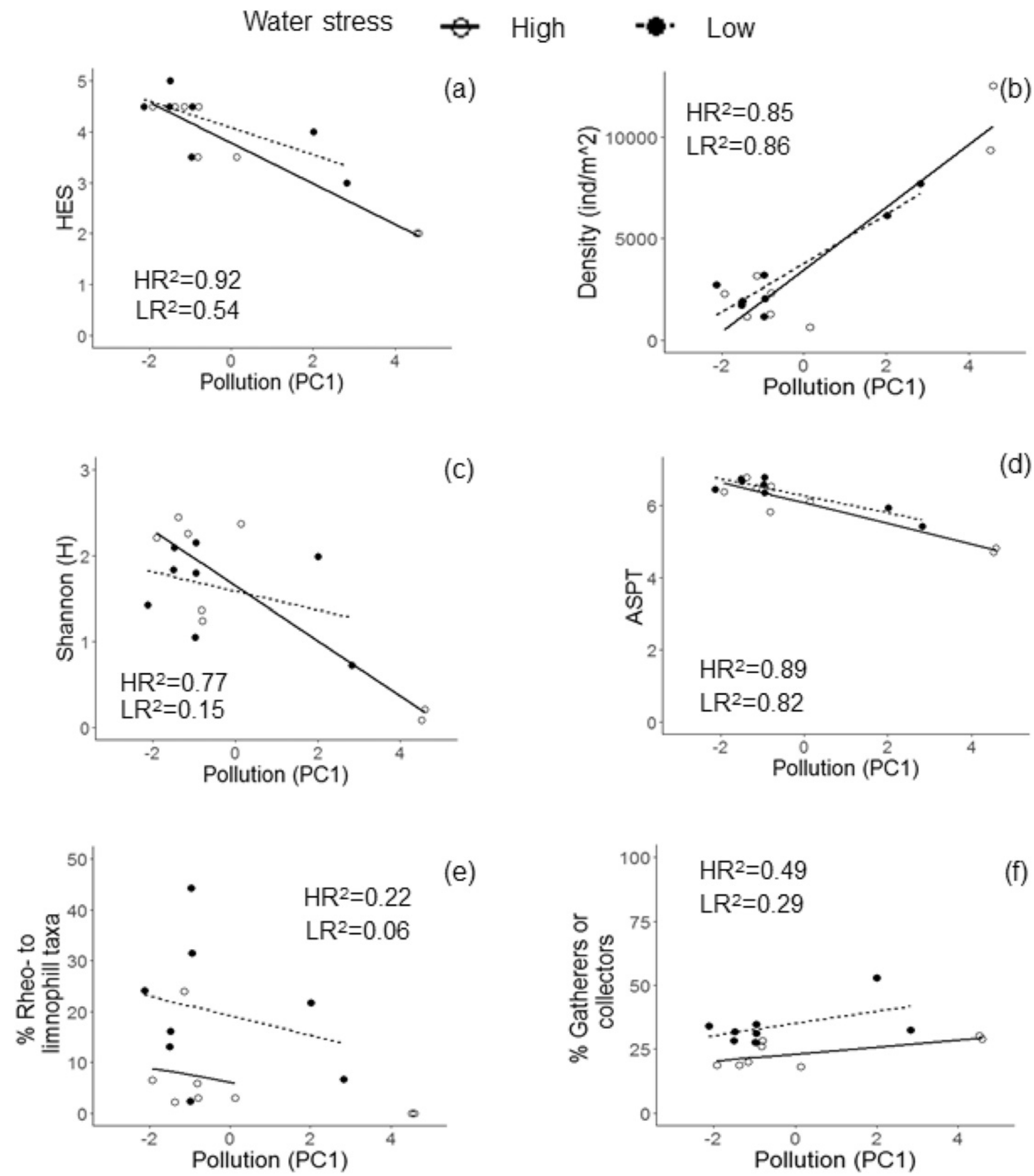

Fig. 4. Graphical representation of trends of selected biotic metrics of macroinvertebrates that significantly responded to water stress and pollution (Table 2 for statistics). HES is the biological quality index for the group, ASPT is the average score per taxon. Solid line and open circles correspond to high water stress, dashed line and filled circles correspond to low water stress. R^2 are only indicative of the fit of each regression line to the corresponding data. HR^2 corresponds to high water stress line and LR^2 to low water stress line.

stress and pollution explained a lower variation in assemblage patterns, with the increasing shared effect rendering the explanation inconclusive. The other biological groups presented responses associated to longer time periods; macrophyte communities responded after 60 days and macroinvertebrates after 45 days (Table 3). Further increasing periods of time resulted in increased shared variation and negative variations of independent groups of variables in macroinvertebrates. Fish presented two periods in time where water stress was relevant, i.e., after 15 days and after 60–75 days. In between, the shared effect increased, making harder the disentanglement of the two groups of variables in explaining assemblage patterns (Table 3).

4. Discussion

Overall, our results indicate a strong, though dissimilar, response of all biotic groups to pollution and water stress. Pollution, and specifically land use, shaped community patterns of all biotic groups. Water stress affected specific functional groups of biota, such as strongly attached diatom taxa, filamentous algae, macroinvertebrate detritus feeders and rheophilic fish species. Fish metrics presented the most pronounced response to the combination of the two stressors. Furthermore, water stress appeared to have a distinct temporal effect on the different biotic groups, as was expected based on their different physiology. Diatom and fish assemblages presented a fast response to water stress (15 days), followed by macroinvertebrate communities (45 days). Macrophyte communities presented the slowest response (60 days), with fish assemblages presenting a second late response to water stress (60, 75 days).

4.1. Effect of water stress and pollution on biotic metrics

The response of diatom metrics to changes in physico-chemical water characteristics, nutrient loading and water flow was similar to

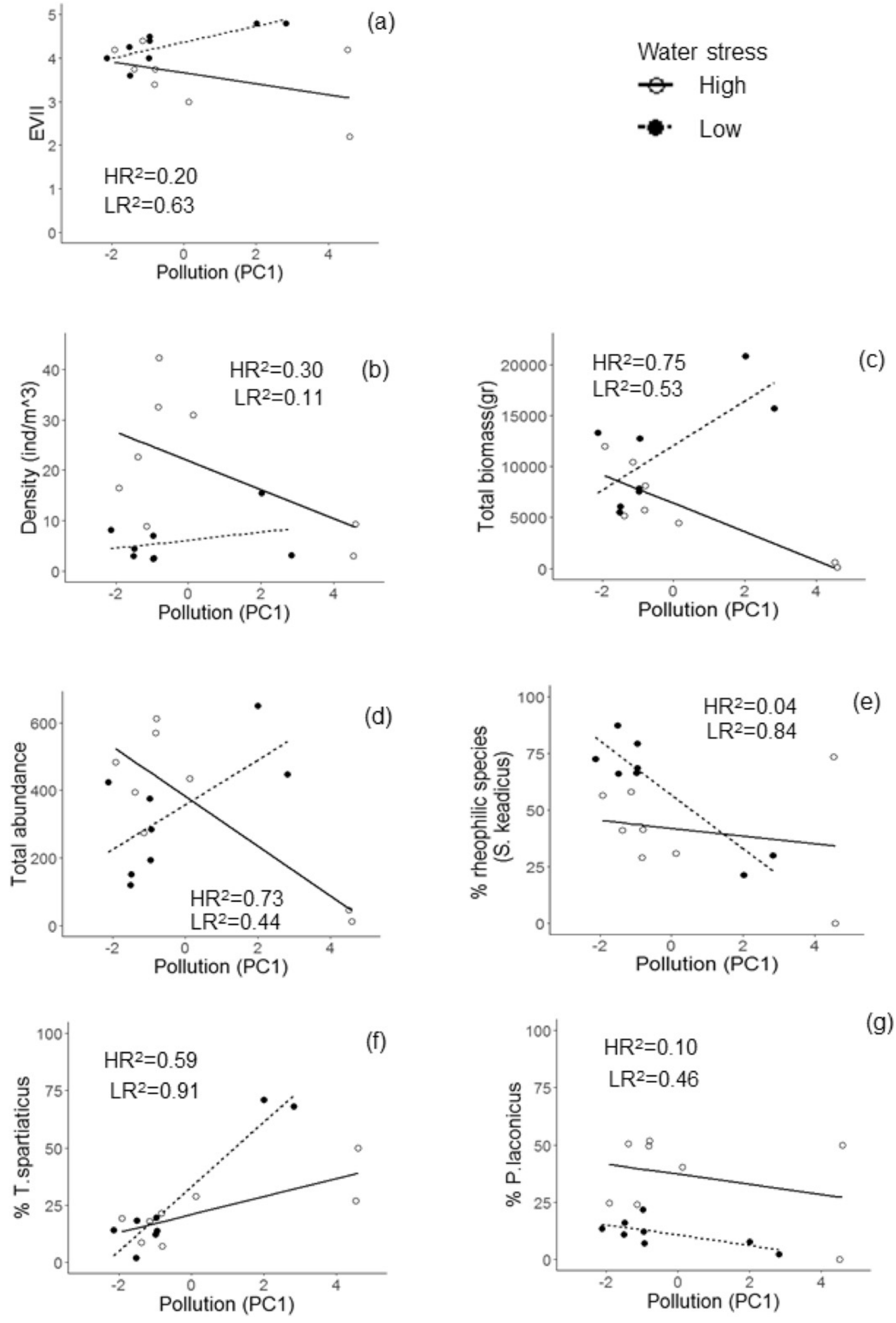

Fig. 5. Graphical representation of trends of selected biotic metrics of fish that significantly responded to water stress and pollution (Table 2 for statistics). EVII is the biological quality index for the group. Solid line and open circles correspond to high water stress, dashed line and filled circles correspond to low water stress. R^2 are only indicative of the fit of each regression line to the corresponding data. HR^2 corresponds to high water stress line and LR^2 to low water stress line.

that reported in other studies on temporary streams (Martínez De Fabricius et al., 2003; Ponsatí et al., 2016). The ecological quality index (IPS) accurately reflected the relevance of pollution on the diatom communities. Diversity metrics were not sensitive either to the pollution gradient or to water stress levels, as these indices in diatoms present a parabolic response to stressors, consistent with the 'Intermediate disturbance hypothesis' (Pandey et al., 2017). At low water stress (and thus high discharge), diatom assemblages were dominated by small adnate species that can be strongly attached to the substratum, resisting shear stress (Sabater, 2000; Tornés et al., 2007). The most common taxa in these conditions were *Achnanthidium* spp., regarded as early colonizers after a disturbance event (Sabater, 2000). At high water stress (and thus lower discharge), thicker biofilms developed, made up by loosely attached taxa (Ponsatí et al., 2016). There was no evidence of a

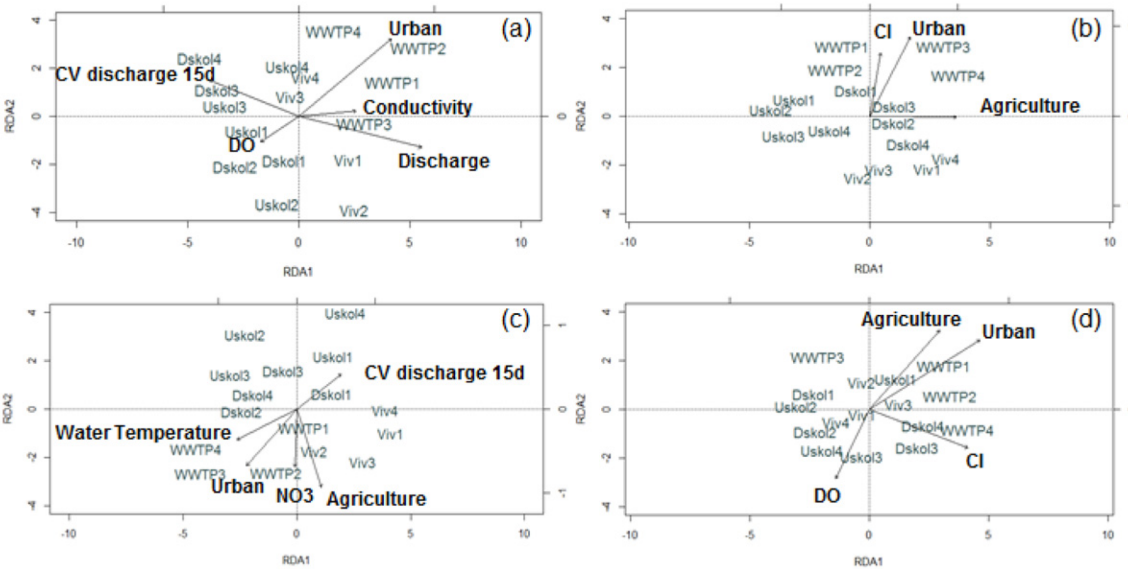

Fig. 6. RDA for variables that significantly contribute in explaining community patterns of diatoms (a), macrophytes (b), macroinvertebrates (c) and fish (d). In gray are the 4 sites; Numbers 1–4 next to the site name indicate the 4 sampling campaigns (June 2014, July 2015, June 2016 and September 2016). Scaling on top and right axis correspond to the environmental variables (arrows).

differential response to pollution at different water stress levels, however different mechanisms were likely in play; thinner biofilms developing at low water stress levels could more easily uptake available chemicals, whereas the lower dilution at high water stress, could intensify the impact of chemicals (Ponsatí et al., 2016).

Macrophyte metrics also responded strongly to pollution, in agreement to previous studies (Hering et al., 2006; Marzin et al., 2012). This was reflected in the ecological quality index's (IBMR_GR) negative response to pollution gradient, as expected (Haury et al., 2006). Helophyte coverage showed a negative response to pollution, agreeing with similar findings of Marzin et al. (2012). Additionally, filamentous bacteria (included in the scoring taxa list for IBMR, though often not categorized as macrophytes) were dominant in the WWTP site, where pollution effects were more acute, due to combined low flow and high water temperature conditions (Phaup and Gannon, 1967). Although numerous studies stress the importance of hydrology for macrophyte communities in rivers (Lacoul and Freedman, 2006; Mackay, n.d.), the results of the current study do not support this, with the exception of filamentous bacteria. This result may be explained by the moderate levels of average flow velocities during the current study, sustaining well developed macrophyte communities (Lacoul and Freedman, 2006; Riis and Biggs, 2003). In addition, it is likely that macrophyte communities are adapted to the intermittent character of the river and the variations in the hydrological regime (Chessman et al., 2008; Westwood et al., 2006), hence small alterations in water velocity may not have any profound effect. Our results also indicate that filamentous algae coverage increases with pollution at low water stress conditions showing a clear effect of nutrients on algal growth (Lacoul and Freedman, 2006) but it decreases when pollution and water stress act together. Frossard et al. (2014) showed that discharge is a key factor for macroalgae structure. In particular, he showed that *Vaucheria* sp. related mainly to high current velocity, whereas *Spirogyra* sp. preferred low flow conditions, a finding that is in agreement with our results. In addition, at high water stress filamentous bacteria can dominate over Spirogyra filaments by becoming attached on them and depriving them of light, as described by Phaup and Gannon (1967). This could be the case

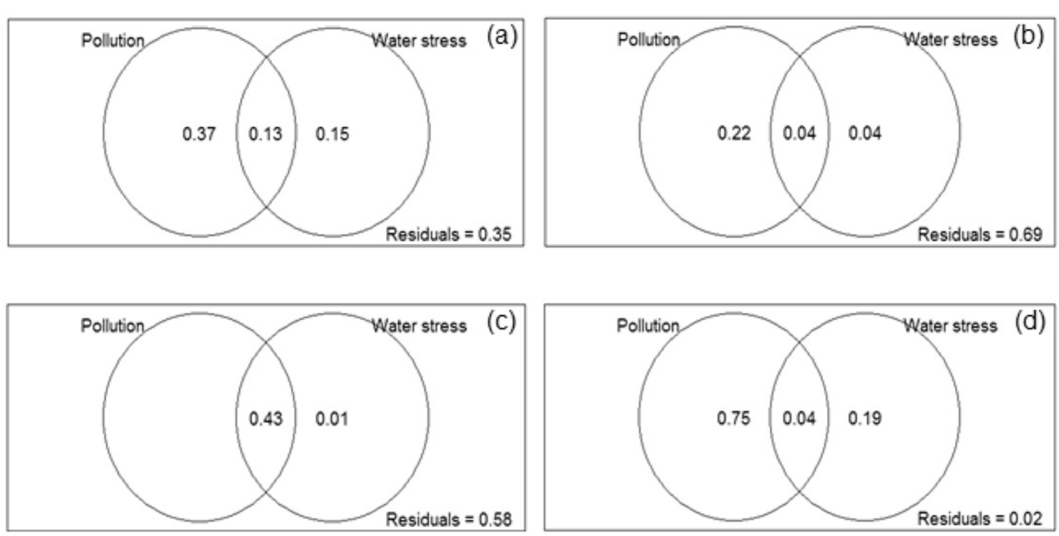

Fig. 7. Variation partitioning showing the explained variation (adjusted R^2) of community patterns in diatoms (a), macrophytes (b), macroinvertebrates (c) and fish (d) of each group of variables. Pollution corresponds to the environmental variables, Water stress corresponds to the hydrological variables. Values <0 are not shown.

Table 3
Differences in explained variation (adjusted R^2) in community patterns over different time-periods for the four biotic groups. Pollution variables were held constant while for water stress variables, different time-series data (15 to 90 days) of simulated discharge were changing in variation partitioning analysis. Pollution|Water stress indicates the shared effects of the two groups of variables. Residuals is the unexplained variation, and is calculated after subtracting the sum of all explained variation from 1. Negative values indicate strong negative correlations between variables or group of variables.

Biotic group	Time (days)	Pollution	Pollution|Water stress	Water stress	Residuals
Diatoms	15	0.37	0.13	0.15	0.35
	30	0.30	0.19	0.13	0.38
	45	0.11	0.38	0.01	0.49
	60	0.15	0.35	0.01	0.49
	75	0.24	0.25	0.11	0.39
	90	0.11	0.38	−0.13	0.63
Macrophytes	15	0.22	0.04	0.04	0.69
	30	0.01	0.25	−0.14	0.88
	45	0.07	0.19	−0.10	0.84
	60	0.30	−0.04	0.10	0.63
	75	0.22	0.05	0.05	0.68
	90	0.17	0.10	−0.01	0.74
Macroinvertebrates	15	−0.02	0.43	0.01	0.58
	30	0.28	0.13	0.14	0.45
	45	0.28	0.14	0.19	0.38
	60	−0.18	0.59	−0.26	0.84
	75	−0.03	0.44	−0.08	0.67
	90	−0.20	0.61	−0.28	0.87
Fish	15	0.75	0.04	0.19	0.02
	30	0.65	0.14	0.11	0.11
	45	0.54	0.24	0.03	0.18
	60	0.78	0.01	0.18	0.04
	75	0.69	0.09	0.18	0.04
	90	0.65	0.14	0.06	0.15

in our study, where at high water stress and high pollution levels, filamentous algae coverage stayed low whereas filamentous bacteria coverage increased.

Benthic macroinvertebrate metrics in temporary rivers and in Evrotas specifically, are known to react to pollution and water stress (Karaouzas et al., 2011; Kalogianni et al., 2017). The results of the current study strongly confirmed the above outcomes, as the ecological quality index (HES) reflected the pollution gradient and was further decreased when water stress was higher. Taxa diversity (Shannon index) also decreased but macroinvertebrate density increased when pollution levels increased and low flow conditions intensified. This tendency was also observed in other studies (e.g. Arenas-Sánchez et al., 2016; Datry et al., 2016); for example, Acuña et al. (2005) found that slight flow reduction resulted in an increase of macroinvertebrate density but reduced biodiversity.

Macroinvertebrate taxon richness decreases as flow intermittency increases, this decrease being more pronounced at high water stress at the polluted reaches (Kalogianni et al., 2017). During the contraction phase, invertebrates with low dissolved oxygen requirements and pool-like strategies are dominant (Acuña et al., 2005; Bonada et al., 2007a; García-Roger et al., 2013). In the current study, high water stress decreased the proportion of rheo- to limnophil taxa whereas other groups were only affected by pollution, with the proportion of rheophil taxa decreasing and that of limno- to rheophil taxa increasing following an increase in pollution levels. Furthermore, an increase in the abundance of miners, parasites and detritus feeders (i.e. gatherers or collectors), was observed as pollution levels increased. Overall, collectors are more abundant in temporary streams than in perennial and the number of predators increases in the remaining wet areas during the contracting phase (Sabater et al., 2006; Bonada et al., 2007b).

Fish responded to water stress, while their response to pollution was related to the level of concomitant hydrological perturbation. At low water stress, fish assemblage attributes presented an upstream-downstream gradient, confirming similar trends previously reported for the Evrotas River fish fauna (Vardakas et al., 2015) and agrees with fish community patterns reported in other Mediterranean streams (Vila-Gispert et al., 2002; Clavero et al., 2005). This gradient mainly reflected the spatial arrangement of fish assemblages and the longitudinal increase in the carrying capacity of the river (Vardakas et al., 2015; Kalogianni et al., 2017), rather than the progressive chemical perturbation, through agriculture and urbanization. At high water stress, however, this trend was reversed, with density, abundance and biomass decreasing with increasing pollution, thus reflecting the progressive chemical impairment of the sites along the Evrotas. Previous studies have shown a significant response of fish metrics to eutrophication/organic pollution gradient and suggested that fish are most strongly affected by oxygen depletion following organic pollution (Hering et al., 2006). Effects on fish abundance and biomass were shaped by the combined effect of water stress and pollution. The combination of natural flow reduction and water abstraction in Mediterranean rivers can exacerbate the effects of pollutants on fish assemblages, by diminishing their dilution capacity (Gasith and Resh, 1999; Figuerola et al., 2012; Kalogianni et al., 2017). A recent study conducted at a pan European scale showed that 70% of combined stressors acted additively or synergistically on fish assemblages (Schinegger et al., 2016).

In the current study, when functional metrics were used at low water stress, the percentage of the rheophilic *S. keadicus* followed a decreasing longitudinal gradient, and that of the limnophilic *T. spartiaticus* an increasing gradient mainly reflecting a natural spatial longitudinal pattern from the headwaters to the river outflow (Vardakas et al., 2015). At high water stress however, both species abundance ceased to follow a gradient, indicating a homogenizing effect of high hydrological perturbation and pollution to the fish fauna. Under such conditions, fish are subject to both the hydrological disturbance and its consequences on the water quality that can jointly cause significant and consistent variation in fish composition and integrity in Mediterranean streams (Matono et al., 2012). Notably, only the small bodied *P. laconicus* presented higher abundances at high water stress possibly related to a shift to faster flowing habitats as recently shown at the reach scale (Kalogianni et al., 2017).

4.2. Effect of water stress and pollution on community matrices

Diatom assemblages' response to water stress indicated that they could be a suitable group for detecting changes in water levels, at least in temporary streams, in contrast to Hering et al. (2006), who acknowledge that diatoms are good indicators of eutrophication and pollution levels but cannot detect hydromorphological degradation. Furthermore, although many predictors of diatom assemblage patterns have been considered, additional variables related to spatial patterns (Soininen, 2007; Tornés and Sabater, 2010) and grazing (Lange et al., 2011) could be applied to further explain observed diatoms patterns.

Macrophyte communities were driven by urban and agricultural land uses and Cl^-, implying a diffuse and point source pollution gradient playing a significant role. The effects of land uses on water quality and aquatic biota in streams have been reported by previous studies (Lenat and Crawford, 1994; Johnson and Hering, 2009). Land use effects were stronger in the WWTP site, where pollution was more intense, whereas in Uskol the small share of agriculture had a small effect on macrophytes. Habitat degradation related to land uses can also affect macrophyte communities particularly in intermittent Mediterranean catchments (Hughes et al., 2009). However, the low explained variation indicates that other factors could be affecting macrophytes such as geomorphology, light availability, substrate characteristics or life-history characteristics of species (Lacoul and Freedman, 2006).

Macroinvertebrate community structure and composition variation occurring at acute water stress in Evrotas has been attributed to habitat alteration and change in water physico-chemistry, i.e. water temperature increase (Kalogianni et al., 2017), as decreases

in water availability are usually associated to increased water temperatures and decreased dissolved oxygen levels. In the current study, reaches influenced by pollution and those by water stress were clearly separated by the ordination diagram. For example, in the most polluted reach (WWTP site), high water temperature, urbanization and discharge variability showed to be major determinants of community structure and composition. In contrast, the low agricultural cover and nitrate concentrations were associated with communities of Dskol and Uskol sites.

Fish communities where mainly formed by their response to urban and agricultural land uses as well as lower D.O. values, as these environmental variables (indicative of pollution) revealed the highest contribution in explaining patterns of fish assemblages based on variation partitioning. Additionally, fish communities presented the highest explained variation by water stress among biotic groups, demonstrating that fish communities are also highly affected by hydrological degradation. A recent study conducted in the Evrotas River indicated that the cumulative effects of low water quality and high water stress can have a very deleterious effect on the Evrotas fish fauna (Kalogianni et al., 2017).

4.3. Temporal differential effects of water stress

Diatoms, compared to other biotic groups, have a large reproductive output and small generation times, allowing them to react faster to hydromorphological and chemical changes (Biggs, 1996). This was evident in the increased explained variation from the water stress component in variation partitioning analysis in short time periods (15 days). The change in the assemblage structure due to water stress detected in only a two-weeks' time was consistent to field experiments elsewhere (Davie et al., 2012).

Macrophytes, in contrast, presented the stronger response to water stress after a period of 60 days, as evidenced by the highest explained variation at this time period (10%), which was still rather low. The results of this study are in line with those of Riis et al. (2008) who examined the composition of macrophyte communities across four flow regime types of lowland rivers and found that it was not strongly correlated to hydrological variables. On the other hand, Frossard et al. (2014) reported that the discharge of the 5 previous days had a significant structuring effect on macroalgal communities.

Macroinvertebrates were mostly affected by water stress after a period of 45 days, as changes in benthic communities can be gradual (see Fig. 2 in Arenas-Sánchez et al., 2016). At 15 days and after 45 days, the shared effect of both stressor groups had a higher effect on the observed community variability than the influence of each stressor separately. This increased correlation between the two stressors in macroinvertebrate communities is in agreement with previous studies of four Iberian river basins (Sabater et al., 2016).

Fish mostly reacted to water stress after 15 days and after 60–75 days, with these two periods possibly related to species specific mortality patterns during drought. More specifically, the two time periods may reflect the initial loss of the rheophilic species and the subsequent collapse, with the progression of drought, of the more resilient limnophilic species (Vardakas et al., 2017b). This variation in mortality patterns possibly reflects differences in the ecological requirements and life-history characteristics of the Evrotas native species (Vardakas et al., 2017b).

4.4. Implications for management

The increasing observed and projected shifts in flow regime to intermittency that is associated to increased water use and climate change dictates changes in the management of temporary rivers that should include the development of new or modified methods to assess their ecological status (Leigh et al., 2016). In this context, our results highlight the importance of considering both stressors (pollution and water stress) when trying to make decisions over the management of intermittent rivers. The complexity of these systems could result in unsuccessful outcomes when considering only one stressor. Furthermore, the time over which water stress acts on the communities should be also considered, since our results indicate different response times of the different biotic groups. More specifically, in regard to diatoms, the biological quality metrics applied were only affected by pollution levels. Therefore, further consideration should be given to adapting existing metrics to water stress levels, as these affect the overall response of the diatom assemblage structure. In regard to macrophytes, the results of this study showed that management plans should focus on point-source pollution mitigation and nutrient limitation from agricultural land use, as these appear to be the main factors affecting macrophyte communities. Additional attention should, however, be given to hydrological factors, since water stress seems to intensify the impacts of pollution on macrophytes, possibly causing unexpected ecological outcomes and implications on the successful integration of management plans. The response of benthic macroinvertebrates to pollution and general degradation is well known. When these are combined with water stress (natural and/or anthropegenic) these effects intensify, as shown in other similar studies worldwide. However, since the effects of water stress on benthic fauna may become evident at a later stage, biomonitoring of temporary streams should involve metrics of BQEs that can be utilized as early warnings of water stress. In regard to fish, structural fish metrics, such as total abundance or biomass may reflect the impairment by chemical stress along an intermittent river only under conditions of high water stress. Functional metrics, however, such as the percentage contribution of rheophilic/limnophilic species may have more localized application, due to their differential response spatially.

5. Conclusions

In the present study, two different types of stressors yielded different responses of the biotic groups of a temporary river. Using biotic metrics commonly applied in river studies, our results indicate that primary producers (diatoms and macrophytes) and macroinvertebrates were mostly affected by pollution, whereas fish by water stress and the combined effect of the two stressors. Water stress also affected specific functional groups of biota, such as strongly attached diatom taxa, filamentous algae and bacteria, macroinvertebrate detritus feeders and rheophilic fish species. Community patterns were mainly affected by environmental variables depicting pollution; while in the case of fish this could reflect a river longitudinal fish population gradient than an actual response to pollution.

The studied biotic groups presented also differences in temporal responses to water stress, with diatom reacting faster due to their small generation times (15 days), followed by macroinvertebrates (45 days) and macrophytes (60 days). Fish reacted both fast (15 days) and slower (60 and 75 days), possibly related to variation in species mortality during desiccation. Overall, our study highlights the importance of considering both stressors (pollution and water stress) as well as the temporal component of biotic responses, when taking management decisions for temporary rivers.

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